CORRELATION OF PREOPTIC NEURONAL ACTIVITY WITH SPONTANEOUS AND INDUCED CORTICAL EEG CHANGES

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Summary : Preoptic area has neurons which change their firing rate along with spontaneous alterations of the cortical EEG between synchronization and desynchronization. The cortical EEG synchronization and desynchronization could be induced by stimulation of the caudal and the rostral brain stem respectively. This study was aimed at finding out whether stimulation of the brain stem structures produce the same change in the unit activity as that occurring during spontaneous alteration of the EEG. Thc changes in unit activity showed some concordance and dissociation between spontaneous and induced EEG alterations. The possible interaction of inputs from cortex and brain stem at the level of the preoptic area is discussed,

Key words : EEG caudal brain stem rostral brain stem electrical stimulation unit activity preoptic area

INTRODUCTION

The preoptic area (POA) is involved in alterations in the cortical EEG and sleep-wakefulness (9, 12, 17, 20, 21, 22). A large percentage of neurons of the POA alter their firing rate with spontaneous changes in the cortical EEG (5, 6). Changes in the POA neuronal activity are likely to be related to some processes causing changes in the cortical EEG. It is possible that alterations in POA neuronal activity are brought about by inputs to this area from two brain structures which are involved in alterations in the cortical EEG. Certain regions in the rostral brain stem reticular formation (RBS) and caudal brain stem reticular formation (CBS) have desynchronizing (16) and synchronizing (13) influences respectively, on the cortical EEG These ascending influences from the brain stem reticular formation may also project to the POA and bring about alterations in the POA neuronal activity, along with the changes in the cortical EEG The available anatomical evidences for

*Present addres : *School of Life Sciences, Jawaharlal Nehru University, New Delhi-110 067 projections from the brain stem reticular formation to POA (4, 15, 23) also support this possibility. Isolated studies have shown that low frequency (LF) stimulation of CBS, which produced synchronization (S), mostly induced an increase in the firing rate of POA neurons (8, 14). High frequency (HF) stimulation of RBS, which produced desynchronization (D), predominantly induced reduction in the firing rate of POA neurons (8, 11). In order to understand the possible complicated relationship, which the brain stem reticular structures may have with POA neurons, this study was undertaken in which changes in the POA neuronal activity during spontaneous and brain stem stimulation induced alterations in the cortical EEG were studied.

MATERIAL AND METHODS

Experiments were conducted on *encephale isole* cats of either sex, weighing between 2.0 and 3.5 *kg*. All the surgical procedures were carried out under ether anaesthesia (6). Local anaesthetic, Marcaine (Bupivacaine hydrochloride, Sarabhai Chemicals, India) was injected into all the pressure points and incisioned margins. The animals were flaxedilised (Gallamine triethiodide, May & Baker, India) and artificially ventilated. The repetition rate of Flaxedil and Marcaine, adjustment of respiratory volume, maintenance of rectal temperature and prevention of distress to the animals were identical to those described earlier (6). After the animals recovered from the effect of ether anaesthesia, one of the stimulating electrodes was stereotaxically lowered and fixed at a S inducing point of the caudal brain stem (CBS) as described earlier (13, 14). S inducing points were located around the nucleus reticularis gigantocellularis (18) within the gigantocellular and magnocellular tegmental fields (1). The other stimulating electorde was fixed at a D inducing structure in the rostral brain stem (RBS) within the formation reticularis mesencephali (11, 18) in the central tegmental field (1) Single neuronal extracellular activity from the POA was picked up from the area lying within the stereotaxic coordinates of A 13.0 to A 15.0, L 0.5 to L 1.5 and H - 1 to H - 4.5 (18). The unit activity was recorded on one of the channels of the polygraph along with the bipolar cortical EEG which was recorded on another, employing the technique described earlier (6). The relationship between the spontaneous change in the EEG and the POA unit firing rate was studied by recording them simultaneously for about 30 min (6). Thereafter the D and S were repeatedly (3-6 times) elicited by the stimulation of the RBS (100 Hz, 0.4 ms and 200-450 μA) and the CBS (6 Hz, 0.4 ms and 220-800 μA), respectively for 10-15s. The effects of these stimulations on the cortical EEG and the POA unit activity were recorded on the polygraph (8, 11, 14).

All the data concerning the unit activity were analysed statistically (6, 8, 11, 14). The significant changes (at the level of 5%) are mentioned here as increased or decreased firing rate. The EEG changes between S and D and their temporal relationship with simultaneous changes in the POA unit activity were visually assessed (6, 8, 11, 14). At the end of the experiments the stimulating and the recording sites were confirmed histologically (6, 11).

RESULTS

Stimulation of the CBS at LF produced stimulus bound S during the period of stimulation, but during the post-stimulatory period, there was no change in the EEG, except in 3 cases where there were D. The S elicited by LF stimulation of the CBS had all the characteristic features describod earlier (13). Stimulation of the RBS at HF induced D which outlasted the period of stimulation.

Out of the 31 neurons of POA studied, 21 showed changes in their discharge rates along with spontaneous alterations in the cortical EEG between S and D. They are described, for convenience, as EEG related neurons. The EEG related neurons consisted of one group of 12 neurons which showed decreased and another group of 9 neurons which showed increased rate of firing during D phase, as compared to S phase. The remaining 10 neurons, which did not show any alteration in their firing rates during spontaneous changes between S and D, formed the third group and were termed as EEG unrelated neurons. The responses of these groups of neurons during S and D induced by the stimulation of the brain stem are described below.

1. *Stimulus induced effects on neurons showing decreased firing rate with spontancous EEG desynchronization :* Out of the 12 neurons of this group, the effect of stimulation of the RBS was studied on 11 while the effects of CBS could be studied on 5 (Table I). All the influenced

TABLE I : Number of POA Neurons showing various effects on stimulation of RBS and CBS.

Relationship with spontaneous EEG changes	*Effect of RBS stimulation*					*Effect of CBS stimulation*				
	T	*S*		*PS*		*T*	*S*		*PS*	
		E	*I*	*E*	*I*		*E*	*I*	*E*	*I*
Decreased firing with D	11	0	9	0	6	5	0	0	0	3
Increased firing with D	7	1	4	4	0	8	0	1	0	0
EEG unrelated	7	2	1	2	0	7	6	0	0	0

D — EEG desynchronization, RBS - rostral brain stem reticular formation, CBS - caudal brain stem reticular formation, T - total number of neurons, E - excitation, I - inhibition.

S — during the period of stimulation, PS - during the period of post stimulation.

neurons showed reduced firing rate during D induced at stimulatory and poststimulatory periods (Fig. 1) The number of neurons showing decreased firing rate with induced D was statistically significant. During the poststimulatory period the decreased firing rate of 4 out of 6 neurons after RBS stimulation and 2 out of 3 neurons after CBS stimulation persisted as long as EEG remained desynchronized.

Discharge rates of POA neurons of this group were not affected during the period of S induced by stimulation of CBS.

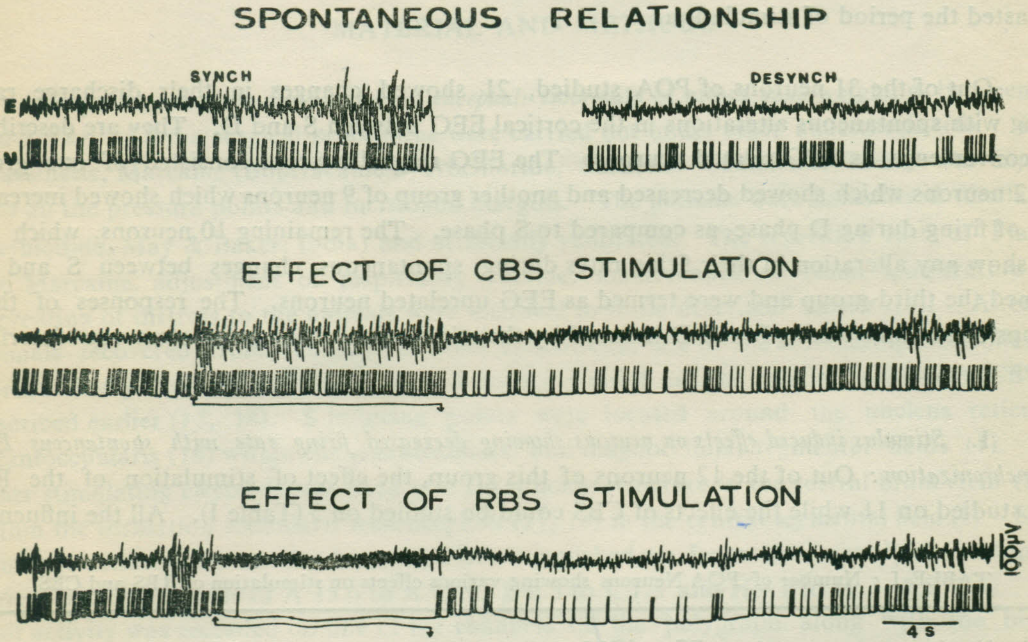

Fig. 1 : The figure shows simultaneous recording of cortical EEG (E) and activity from one POA neuron (U). The upper strips show increased firing of the neuron during spontaneous synchronization (SYNCH) as compared to desynchronization (DESYNCH). Effect of stimulation of caudal brain stem (CBS) at low frequency and rostral brain stem (RBS) at high frequency are shown in second and third strips respectively. The period of stimulation is underlined with arrows on either end indicating onset and termination of stimulation. There was no statistically significant change in the neuronal firing rate during EEG synchronization produced by LF stimulation of CBS though the firing becomes more regular during this period.

2. *Stimulation induced effects on neurons showing increased firing rate during spontaneous EEG desynchronization :* Of the 9 neurons of this group, the effect of stimulation of the RBS was studied on 7, while the effect of stimulation of the CBS was studied on 8 (Table I). Most of

the influenced neurons showed reduced rate of firing during D produced at the time of stimulation of the RBS. On the other hand, during the poststimulatory D, all the influenced neurons showed increased rate of firing. The increased discharge rate of all these neurons continued as long as the poststimulatory EEG remained desynchronized (Fig. 2). The number of neurons showing a decrease in firing rate during the period of stimulation, an increase during the poststimulatory period, and an increased firing rate temporally correlated with induced D during the poststimulatory period were significant.

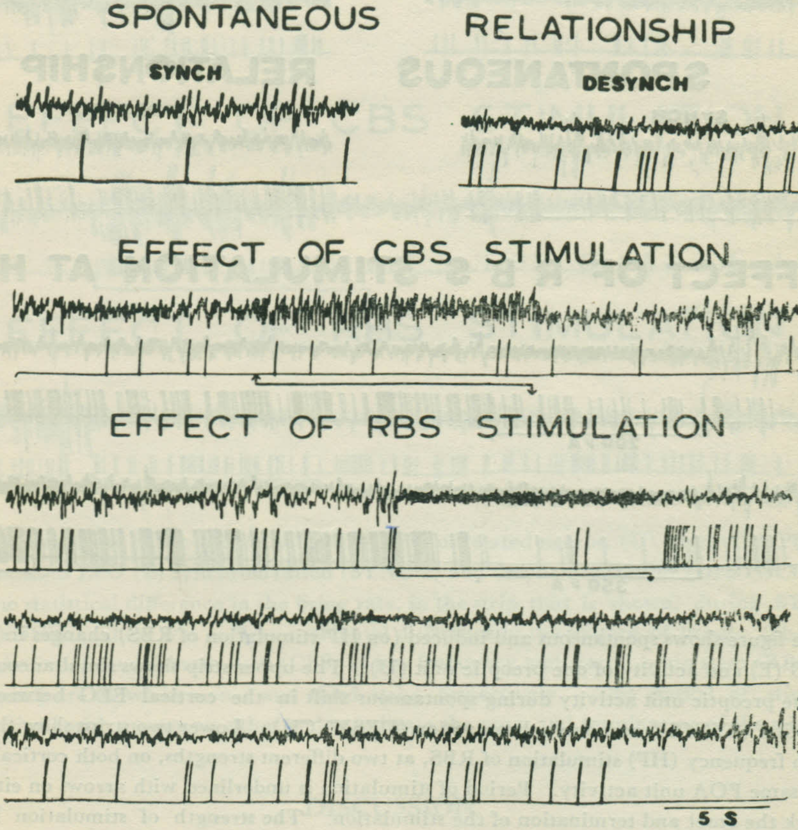

Fig. 2 : The figure shows simultaneous record of cortical EEG (E) and activity from one POA neuron (U). The upper most strips show POA neuronal firing during spontaneous change in the cortical EEG between synchronization (SYNCH) and desynchronization (DESYNCH). Effect of stimulation of caudal brain stem (CBS) at low frequency is shown in second strip. Continuous record of changes in the EEG and the neuronal activity after HF stimulation of rostral brain stem (RBS) is shown in lower three strips (1, 2 and 3). The period of stimulation is underlined and marked with arrows on either end to indicate the onset and termination of stimulation.

There was no decrease in the firing rate during the period of stimulation in one of these neurons, when stimulus strength was reduced to the level just sufficient to induce D. But there was an increase in the rate of firing during the poststimulatory D (Fig. 3). Slightly higher strength of stimulus delivered to the RBS produced decreased rate of firing during stimulation, though the increased rate of firing during the poststimulatory period persisted.

The neurons of this group were generally unaffected during S induced by stimulation of the CBS. They were not affected during the poststimulatory period also.

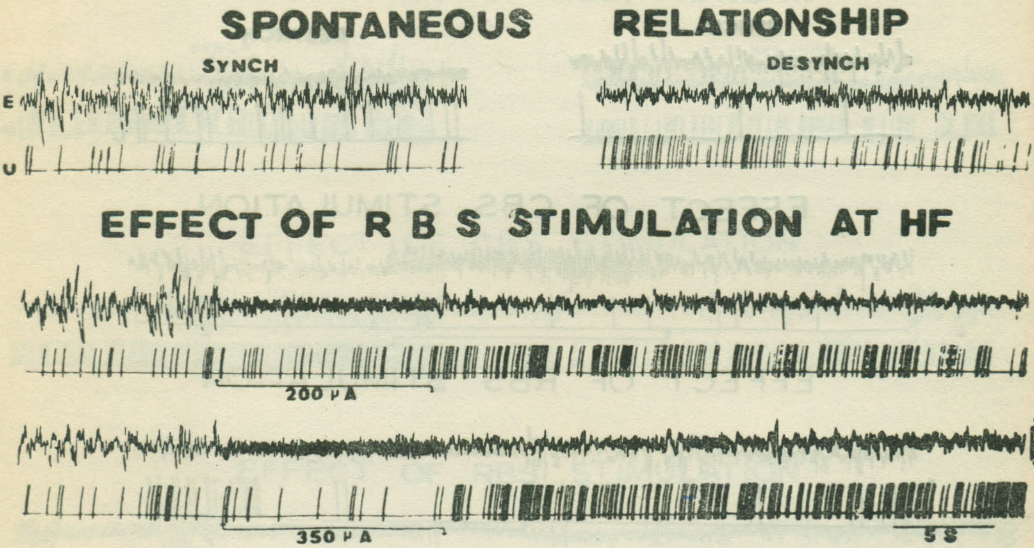

Fig. 3 : The figure shows spontaneous and induced (on HF stimulation of RBS) changes in the cortical EEG (E) and activity of one preoptic unit (U). The upper strip shows simultaneous recording of the preoptic unit activity during spontaneous shift in the cortical EEG between synchronization (SYNCH) and desynchronization (DESYNCH). Lower two strips show the effects of high frequency (HF) stimulation of RBS, at two different strengths, on both cortical EEG and the same POA unit activity. Period of stimulation is underlined with arrows on either ends to mark the onset and termination of the stimulation. The strength of stimulation is indicated below the underlined region.

3. *Stimulation induced effects on EEG unrelated neurons :* The effects of stimulation of the RBS and the CBS could be studied on 7 neurons each, out of the 10 neurons of this group (Table I, Fig. 4) The number of neurons showing altered firing rate during induced D was not found to be significant Also, none of them showed temporal correlation of their discharge with the poststimulatory D. Almost all the neurons of this group showed increased

discharge during the S induced by the stimulation of the CBS (Fig. 4). None of the neurons was affected during the poststimulatory period.

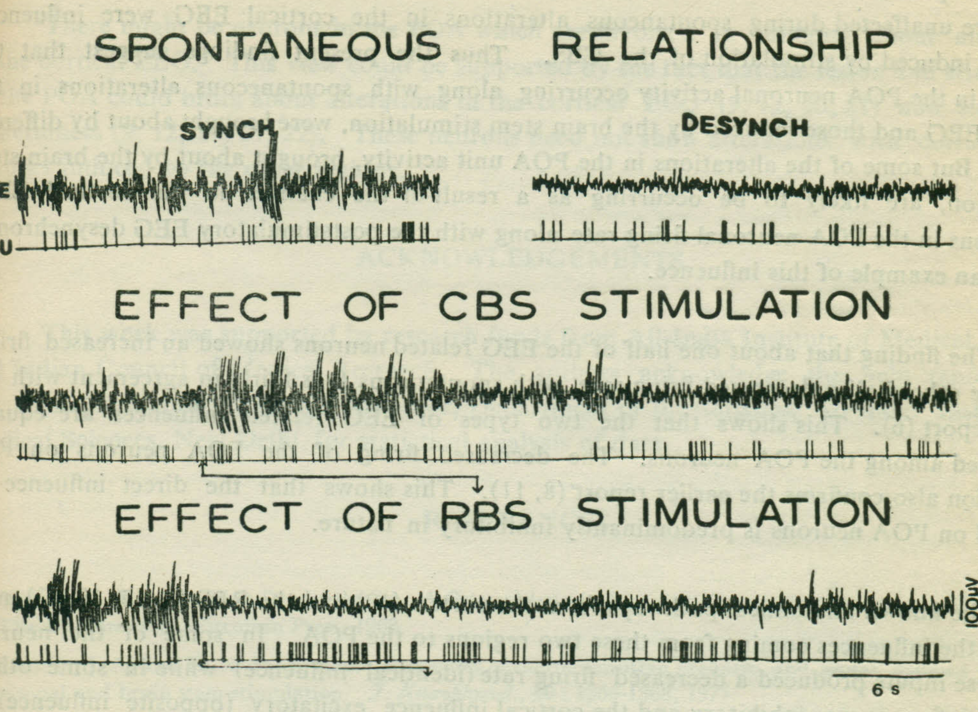

Fig. 4 : The figure shows on top the activity of an EEG unrelated neuron (U) from the POA during spontaneous EEG (E) synchronization (SYNCH) and desynchronization (DESYNCH). There was no statistical difference in the firing rate, in the strip that is shown, during SYNCH and DESYNCH. There was only an incidental difference in the pattern of firing. The effects of stimulation of caudal brain stem (CBS) at low frequency and rostral brain stem (RBS) at high frequency are shown in second and third strips respectively. The period of stimulation is underlined with arrows on either end to indicate the onset and termination of the stimulus.

DISCUSSION

Projections from RBS and CBS to POA have been demonstrated using anatomical and physiological techniques (2, 3, 4, 15, 19, 23). It is well established that D and S of the cortical EEG could be brought about by RBS and CBS stimulations respectively (8, 11, 13, 14, 16). On the basis of these findings it is tempting to suggest that the outputs from RBS and CBS bring about changes in the cortical EEG and POA unit activity simultaneously. The results of this study do not support that contention. Firstly, the EEG related neurons were generally unaffected during S induced by stimulation of the CBS. Secondly, most of the neurons which showed increased firing rate with spontaneous D exhibited a decreased discharge during the period of stimulation of the RBS. Thirdly, most of the neurons whose discharge rates were unaffected during spontaneous alterations in the cortical EEG were influenced during S induced by stimulation of the CBS. Thus the present findings suggest that the changes in the POA neuronal activity occurring along with spontanecous alterations in the cortical EEG and those induced by the brain stem stimulation, were brought about by different inputs. But some of the alterations in the POA unit activity, brought about by the brain stem stimulation, are likely to be occurring as a result of the changes in the cortical EEG. Alterations in the POA neuronal firing rate along with the poststimulatory EEG desynchronization is an example of this influence.

The finding that about one half of the EEG related neurons showed an increased firing, while the other half a decreased firing rate, with spontaneous D is fairly in agreement with the earlier report (6). This shows that the two types of EEG related influences are equally distributed among the POA neurons. The decreased firing of the POA neurons on RBS stimulation also confirms the earlier report (8, 11). This shows that the direct influence of the RBS on POA neurons is predominantly inhibitory in nature.

The known anatomical pathways from the cortex (10) and the RBS (3, 15, 19, 23) may mediate the influences coming from these two regions to the POA. In some of the neurons both these inputs produced a decreased firing rate (identical influence) while in some others the RBS influence was inhibitory and the cortical influence excitatory (opposite influence) in nature. The opposite influence of the second type may be responsible for the different responses obtained during stimulatory and poststimulatory periods on RBS stimulation. Existence of dual influences of opposite nature was further supported by the observation in which the responses could be independently elicited (Fig. 3).

Difficulty lies in explaining the absence of effects on the POA neurons during stimulation induced S. Even the EEG related neurons, which showed alteration along with induced D, did not show similar changes along with induced S. Anatomical (15, 21) as well as physiological (4, 7, 8, 14) studies have shown inputs from CBS to the POA. Physiological study has shown an excitatory input from the CBS to the POA even in the absence of the induced EEG changes (7, 14). It might be possible that the CBS influence on the POA neurons was primarily mediated through its direct input As mentioned in an earlier report (13), the CBS stimulation had to be given on the background of S to induce the stimulus bound S. In this background the EEG related POA neurons might nct be in a position to alter their discharge rates further along with induced S. It would also be wrong to assume that the changes in the EEG induced by brain stem (CBS and RBS) stimulation, or any other manipulation, can be totally comparable to similar EEG changes occurring spontaneously.

There might be neurons in the POA which are involved in bringing about alterations in the cortical EEG. This view could be supported by the fact that the lesion and stimulation of the POA could bring about alterations in the cortical EEG (9, 12, 20, 21) and sleep and wakefulness (9, 12, 17, 21, 22). These neurons need not show alterations with cortical EEG changes induced by the other regions,

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REFERENCES

1. Berman, A. L. *The Brain Stem of the Cat* : A *Cytoarchitectonic Atlas with Stereotaxic Coordinates*. Madison WI, The University of Wisconsin Press, 1968.

2. Boulant, J. A. and H. N. Semieville. Responses of thermosensitive preoptic and septal neurons to hippocampal and brain stem stimulation. *J. Neurophysiol.*, **40** : 1356-1368, 1977.

3. Edwards, S. B. and J. S. De Olmos. Autoradiographic studies of the projections of the midbrain reticular formation : Ascending projections of nucleus cuneiformis. *J. Comp. Neurol.*, **165** : 417-432, 1976.

4. Kaba, H., H. Saito, K. Seto and M. Kawakami. Antidromic identification of neurons in the ventrolateral part of medulla oblongata with ascending projections to the preoptic and anterior hypothalamic area (POA/AHA). *Brain Research*, **234** : 149-154, 1982.

5. Kaitin, K. I. Preoptic area unit activity during sleep and wakefulness in the cat. *Exp. Neurol.*, **83** : 347-357, 1984,

6. Mallick, B. N., G. S. Chhina, K. R. Sundaram, B. Singh and V. Mohan Kumar. Activity of preoptic neurons during synchronization and desynchronization. *Exp. Neurol.*, 81 : 586-597, 1983.

7. Mallick, B. N., V. Mohan Kumar, G. S. Chhina and B. Singh. Responses of preoptic neurons to stimulation of caudal and rostral brain stem reticular structures. *Brain Res. Bull.*, **13** : 353-356, 1984.

8. Mallick, B. N., V. Mohan Kumar, G. S. Chhina and B. Singh. Comparison of rostro-caudal brain stem influence on preoptic neurons and cortical EEG. *Brain Res. Bull.*, **16** : 121-125, 1986.

9. Mc Ginty, D. J. and M. B. Sterman. Sleep suppression after basal forebrain lesions in the cat. *Science*, **160** : 1253-1255, 1968.

10. Mizuno, N., E. K. Sauerland and C. D. Clemente. Projections from the orbital gyrus in the cat. II. To telencephalic and diencephalic structures. *J. Comp. Neurol.*, **136** : 127-142, 1968.

11. Mohan Kumar, V., B. N. Mallick, G. S. Chhina and B. Singh. Influence of ascending reticular activating system on preoptic neuronal activity. *Exp. Neurol.*, **86** : 40-52, 1984.

12. Mohan Kumar, V., S. Datta, G. S. Chhina and B. Singh. Sleep awake responses elicited from medial preoptic area on application of norepinephrine and phenoxybenzamine in free moving rats. *Brain Research*, **322** : 322-325, 1984.

13. Mohan Kumar, V., G. S. Chhina and B. Singh. Mapping of regions in the caudal brain stem that produce stimulus bound synchronization in the cortical EEG. *Exp. Neurol.*, **89** : 295-305, 1985.

14. Mohan Kumar, V., B. N. Mallick, G. S. Chhina and B. Singh Alterations in preoptic unit activity on stimulation of caudal brain stem EEC-synchronizing structures. *Exp. Neurol.*, **89** : 304-313, 1985.

15. Morgane, P. J. and W. C. Stern. Chemical anatomy of brain circuits in relation to sleep and wakefulness. In *Advances in Sleep Research* by E. Weitzman, Vol. I, Spectrum, New York, p 1-131, 1974.

16. Moruzzi, G. and H. W. Magoun. Brain Stem reticular formation and activation of the EEG. *Electroenceph. Clin. Neurophysiol.*, **1** : 455-473, 1949.

17. Nauta, W J. H. Hypothalamic regulation of sleep in rats. An Experimental Study. *J. Neurophysiol.*, **9** : 285-316, 1946.

18. Snider, R. S. and W. T. Niemer. *A Stereotaxic Atlas of the Cat Brain*, Chicago : University of Chicago Press, 1961.

19. Steriade, M., N. Ropert, A. Kitsikis and G. Oakson. Ascending activating neuronal networks in midbrain reticular core and related rostral systems. In *Reticular formation revisited* by J. A. Hobson and M. A. B. Brazier. Raven Press, New York, p 125-167, 1980.

20. Sterman, M. B. and C. D. Clemente. Forebrain inhibitory mechanisms : cortical synchronization induced by basal forebrain stimulation. *Exp. Neurol.*, **6** : 91-102, 1962.

21. Sterman, M. B. and C. D. Clemente. Forebrain inhibitory mechanisms : Sleep patterns induced by basal forebrain stimulation in behaving cat: *Exp. Neurol.*, **6** : 103-117, 1962.

22. Sterman, M. B., T. K. Knauss, D Lehmann and C. D. Clemente. Alteration of sleep patterns following basal forebrain lesions. *Fed. Proc.*, **23** : 209, 1964.

23. Sutin, J. and R. L. Mc Bride. Limbic and brain stem connections of the hypothalamus. In : *Handbook of the Hypothalamus : Anatomy of the Hypothalamus* by P. J. Morgane and J. Panksepp. Marcel Dekker, New York, p. 555-592, 1979.